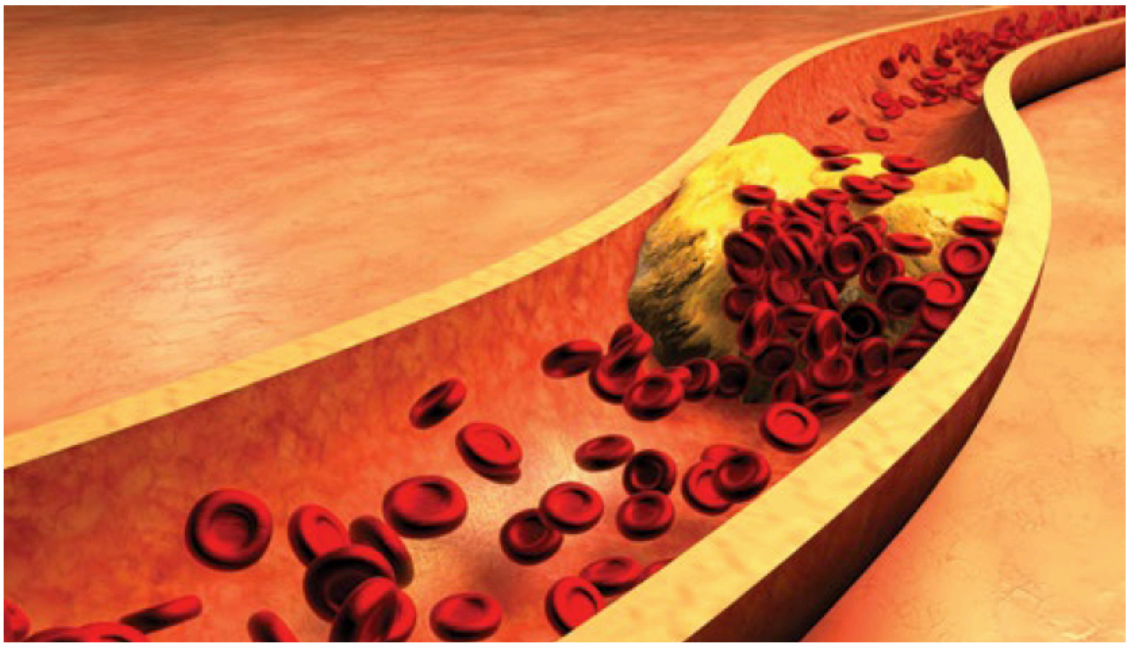
Hypercholesterolemia MiniLab

Student's Guide

Cat# M3051

Version 091922



A special thank you to Crystal McDowell for her contribution that made this MiniLab possible.

Table of Contents

Laboratory Safety

1. Wear lab coats, gloves, and eye protection as required by district protocol.
2. Use caution with all electrical equipment such as PCR machines and electrophoresis units.
3. Heating and pouring molten agarose is a splash hazard. Use caution when handling hot liquids. Wear eye protection and gloves to prevent burns.
4. Wash your hands thoroughly after handling biological materials and chemicals.

Introduction - The First Signs

"Hi! Sorry to keep you waiting, Ms. Thomas. And this must be Amber. My name is Dr. Patel, but you can call me Dr. P. So, what brought you in today, Amber?" Dr. Patel said, smiling at the seven-year old child.

"I have these bumps on the back of my foot, Dr. P, and yellow spots on my elbows and knees. They don't really hurt but they can be itchy," Amber said as she gave Dr. Patel a fist bump.

"Let's take a closer look. Hmm. I see what you are talking about. Are the spots tender, too? When I touch it, does it feel different or hurt a little?" Dr. Patel asked.

"Yeah, I guess," Amber said.

"Can I also look into your eyes, Amber?" Dr. Patel asked.

"Sure, Dr. P!" Amber smiled.

Dr. Patel looked carefully into Amber's eyes. She saw exactly what she expected, the beginnings of corneal arcus, a white-grey deposit around the cornea. Dr. Patel took another look at Amber's Achilles tendon and noticed that there was slight bilateral enlargement of both tendons.

"Amber, why don't you go with Nurse Emily to pick out something from the prize box for being such a great patient and I am going to talk with your mom for a few moments? Then, we may run a few tests to check on your spots, ok?" Dr. Patel asked.

"Sure," Amber said excitedly. Amber and Nurse Emily exited the room.

"Is it something serious, Dr. Patel? You look concerned." Ms. Thomas (Jada) asked.

"Mrs. Thomas, yellowish patches of skin on Amber's elbows and knees are likely what we call cutaneous xanthomas. These skin lesions often result from deposits of lipids, specifically cholesterol, in tissue. This could also explain the nodules on the Achilles tendons which are known as tendinous xanthomas. Lipid deposits can actually become wrapped around the tendon and become subcutaneous. The white-grey deposit along the circumference of the cornea also indicates deposits of cholesterol. While the deposits do not appear harmful at this time, they could point to an underlying cause that could be problematic for Amber. High levels of LDL cholesterol could be caused by a number of factors."

"But Dr. Patel, Amber is seven-years old. How could she have high cholesterol?" Jada, interrupted. "Isn't she too young for that?"

"Usually, yes, but there are some conditions that increase the LDL cholesterol levels in individuals like diabetes, hypothyroidism when the thyroid gland is not producing enough hormones, metabolic conditions, and, although rare, a form of familial hypercholesterolemia. Let's take this one step at a time and first do some blood work to confirm Amber's LDL cholesterol levels. I would also like to take a tissue sample to confirm the xanthomas. Would that be okay?" asked Dr. Patel.

"Yes. I guess. I definitely want to find out if there is an underlying condition we did not know about," Jada said.

Analysis Questions - Obtain, Evaluate and Communicate Information about the case

1. Explain what it means when Dr. Patel uses the term "bilateral" for the enlarged Achilles tendon?

2. Describe why the lesions are called "cutaneous"? Identify the anatomical structure affected by the lesions.

3. Describe why the potential lipid deposits that might intertwine with the tendon are called "subcutaneous"? How does the location of these structures relate to this term?

4. What might cause a person with diabetes to have high LDL cholesterol levels (effect)? What other symptoms (effects) might you expect?

5. What does the "hypo" prefix mean for hypothyroidism?

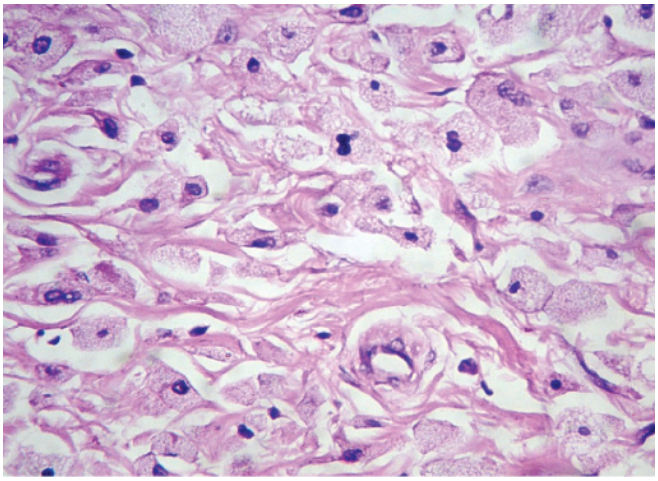
6. What type of life function do thyroid hormones control? Why might a lack of production of these hormones cause a buildup of LDL cholesterol? Look up hypothyroidism. What other symptoms (effects) would you expect?

7. Predict whether familial hypercholesterolemia is an inherited condition or environmentally induced by diet? What does the "hyper" prefix mean?

The Initial Results

Test	Amber's Results	Normal Range	Low, Normal or High
Blood Glucose Level	110 mg/dl	80-140 mg/dl	Normal
LDL Cholesterol	532 mg/dl	Acceptable is Less than 110 mg/dl Borderline is 110-129 mg/dl	Extremely High
Thyroid-Stimulating Hormone (TSH)	2.1 mlU/L	1.7-3.0 mlU/L	Normal
Free T4	1.26 ng/dl	1.12-1.35 ng/dl	Normal
Free T3 (female)	3.9 pg/ml	3.6-4.3 pg/ml	Normal

Histopathology Results



Tissue samples from the xanthoma did show the characteristic foamy macrophages with vacuoles containing lipid droplets within the dermis. Image Source: https://www.jcasonline.com/viewimage.asp?img=JCutanAesthetSurg_2012_5_3_204_101384_u4.jpg

Dr. Patel examined the results of the blood-lipid tests and the hormone levels as well as the histopathology report. It was pretty evident what the diagnosis was likely to be, but Dr. Patel had never seen a case in a child so young. She knew it was rare but possible.

Analysis Questions - Analyze and Interpret Patient Data from the Case

1. Make **claims** about which conditions you can eliminate as possible diagnoses? **Cite evidence** to explain why you reached that conclusion for each condition.

2. Make a **claim** about which condition is likely the appropriate diagnosis? **Cite evidence** to support that claim. Why is this diagnosis alarming?

Potential Diagnosis - Unexpected Prognosis

"But, what do you mean? How could my seven-year old daughter be at risk of a heart attack? She's just too young. I don't understand." Jada was overwhelmed and in shock. She and her husband, Jared, were meeting with Dr. Patel to discuss Amber's test results.

"Let me start at the beginning. First let's talk about cholesterol. Your body needs some level of cholesterol because it is an important component of cell membranes, some hormones, and Vitamin D. Cholesterol is produced by the liver and we can obtain the amount we need from that synthesis process, but we also get cholesterol from the food we eat which is why diet is important. The liver is also important in taking up cholesterol from the bloodstream to remove it from the body. Cholesterol travels through your bloodstream as lipoproteins and there are two types. HDL is what many people call 'good cholesterol.' This high-density lipoprotein actually helps the body to get rid of LDL cholesterol which is known as 'bad cholesterol.' HDL also helps to prevent cholesterol from building up in a person's arteries. LDL is more likely to form the plaques of cholesterol buildup in the arteries leading to atherosclerosis ('hardening of the arteries') and heart disease," Dr. Patel explained.

"But Amber is a relatively healthy child. She is a normal height and weight for her age. Her diet could probably improve, but it is not completely unhealthy. What is causing her levels of LDL in her blood to be so high?," asked Jared, Amber's dad.

"That is an excellent question and is why it is really important for both of you to be here today. There is a genetic condition known as Familial Hypercholesterolemia. It is actually pretty common in the heterozygous form (HeFH). One in 250 individuals actually have this form of the inherited condition. It is an autosomal dominant condition, meaning if you have just one of the mutated variants/alleles of one of the genes, then you will have FH. At the biochemical level, however, it can be described as incomplete dominance or impact the patient differently due to dosage of the protein affected depending on whether the individual has one copy of the mutated gene and is heterozygous or two copies of the mutated gene and homozygous. We suspect that Amber might have a rare form of FH known as Homozygous Familial

Hypercholesterolemia (HoFH) which would mean that she inherited two copies of the mutated allele for the gene, one from each of you. This form of the condition only occurs in about 1 in 250,000 people. If our diagnosis is correct, then it is very important to do what is known as a cascade screening for your family." Dr. Patel paused to allow that information to sink in.

Jada and Jared were stunned. They both knew there was family history of heart disease but they had no idea that it could be connected to a genetic disorder in their family. They assumed it was related to diet alone.

"So, what can we do to find out for sure. Is there a genetic test available? Is that what you mean by a screening? And what can we do to prevent or reduce the risks of heart disease?" asked Jada.

"Remember, Ms. Thomas, how I said we were going to take it one step at a time. I know you have lots of questions and we are going to provide you with information and guidance as we figure out what is causing Amber's LDL levels to be so high. Here is my next suggestion. I would like to set you up an appointment with a genetic counselor. There is a genetic screening test available and the counselor can walk you through more specifics of how this condition is inherited. She will also set up the genetic screening test for you, your husband, and Amber. Do you have any other children?" Dr. Patel asked.

"We have a 10 year old son, Aaron, and we are guardians of our nephew, Cameron, who is four years old," said Jared.

"With your consent, let's test everyone in your immediate household for now and then we will discuss a cascade screening based on those results" explained Dr. Patel.

"Yes, please. We want to know if any of us could possibly be affected and if this condition is what is causing Amber's LDL levels to be so high," replied Jared.

Analysis Questions - Obtain, Evaluate and Communicate Information about the Case

1. If Jada and Jared do have HeFH, what would their genotype be if we use the letter "F"? Remember that a genotype are two letters that represent the versions of a gene a person has inherited from each of their parents. Heterozygous means that Jada and Jared would have inherited one mutated copy of the gene or DNA variant and one normal copy of the gene. Would their genotype be FF, Ff, or ff?

2. If Amber does have HoFH, what would her genotype be?

3. What **causes** high LDL levels to be more harmful than HDL levels?

4. Typically, a genetic screen would only be performed on the individual exhibiting symptoms/ clinical criteria first before testing others in the family. **Evaluate** why this would be a better approach?

5. Why did Jared and Jada relate their family's heart disease (effect) with diet (cause)?

Genetic Screening in the Biotechnology Lab

As a Molecular Genetics Technologist, you will be performing the genetic screening test today for Jared, Jada, Amber, Aaron and Cameron. Below are the protocols used by this lab. Since you are new to your position, follow the directions below exactly and listen to further instructions provided by your supervisor.

Part I: Electrophoresis

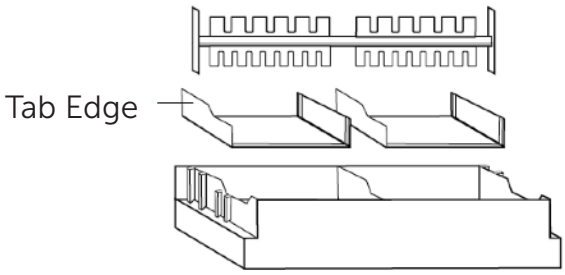
Materials

1 Minione® Casting System
1 MiniOne® Electrophoresis System
1 agarose GreenGel™ cup (0.8 %)
8 DNA sample aliquots
135 mL of running buffer
1 micropipette (2-20µL)
10 pipette tips

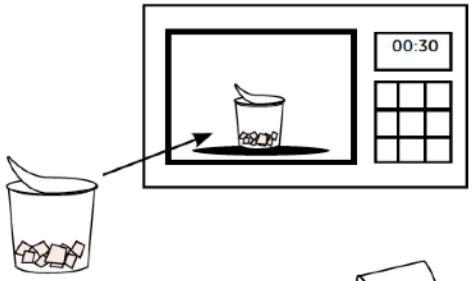
Lane #	Sample Name	Volume
1	MiniOne Universal DNA Marker	10 µL
2	FH Normal (ff) Control	10 µL
3	FH Carrier (HeFH, Ff) Control	10 µL
4	FH Patient (HoFH, FF) Control	10 µL
5	Cameron	10 µL
6	Jada	10 µL
7	Aaron	10 µL
8	Jared	10 µL
9	Amber	10 µL

How to Cast a Gel

1. Place the MiniOne® Casting Stand on a level surface and place gel trays in the two cavities. For proper tray orientation place the tab edge of the tray on the left side. Insert the comb into the slots at the top of the casting stand with the 6-well side facing down.

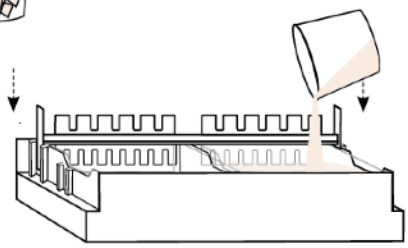


2. **Partially** peel the film off a GreenGel™ cup and microwave for 25-30 seconds. Allow to cool for 15 seconds. DO NOT microwave more than 5 gel cups at a time.



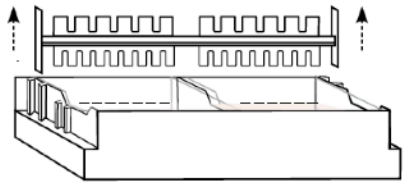
Safety requirement: Adult supervision required if students are handling gel cups!

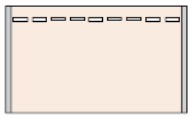
3. One gel cup is for making one agarose gel! Slowly pour the hot agarose solution into a gel tray. Make sure there are no air bubbles in the agarose solution. Let the agarose gel solidify for 10 minutes or until opaque.



DO NOT disturb the gel until time is up.

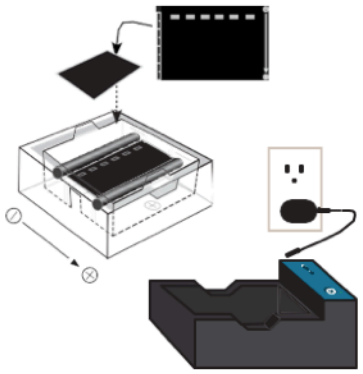
4. Carefully remove comb when gel is ready. Remove gel tray with solidified gel from Casting Stand and wipe off any excess agarose from the bottom of the tray.





How to Load a Gel

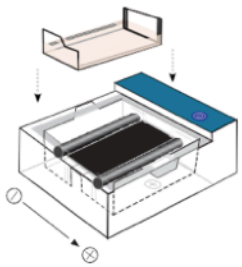
1. Ensure the black viewing platform is in the gel tank. **Make sure the wells are aligned with the marks on the platform on the negative end.**



2. Plug the power supply into the wall and carefully insert the other end into the back of the MiniOne® Carriage.

3. Place the gel tank into the carriage so the carbon electrodes are touching the gold rivets and the tank sits level with the carriage.

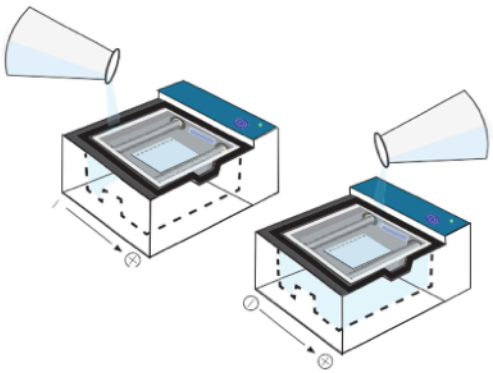
4. Place the gel tray with the gel into the gel tank. The gel tank should not have any buffer in it when putting the gel tray with gel into it.



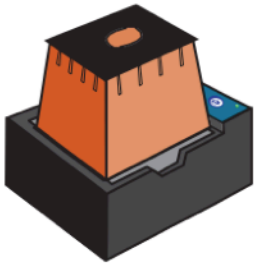
Note: Place gel tank with the gel on the gel tray into carriage **before** pouring buffer in.

5. Turn the low intensity blue LED on by pressing the button on the carriage.

6. Measure 135 mL of TAE running buffer and pour into **one side** of the gel tank. Watch the air push out between the gel tray and viewing platform. Once air has been removed from under the gel tray, pour remaining buffer into the **other side of the gel tank.**

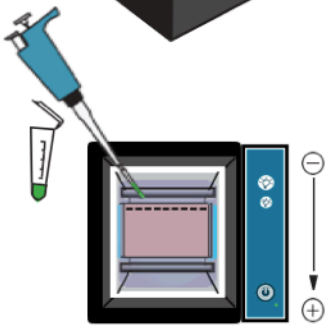


7. Place photo hood on the carriage.



8. Press the power button which should now be a solid green light. If **green light is solid,** turn off the unit and proceed to loading gels.

9. Turn the low intensity blue light on by pressing the button on the carriage to help visualize the wells when loading.



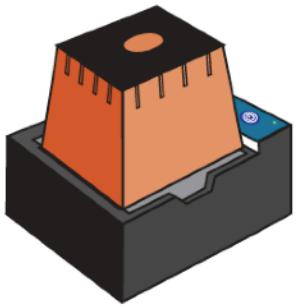
10. Load 10 µL per well. Remember to change pipette tips for each sample. **Load your samples according to the order given in the sample chart.**

Run, Visualize and Capture Image

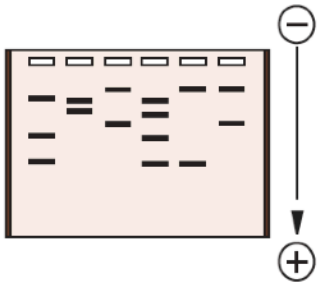
1. Once the gel is loaded, do not move it. Make sure the power supply is plugged in and place the photo hood on the carriage. Turn on the unit by pressing the button. The green LED next to the button will turn on.

   The green power LED will not turn on if:

   - **The tank is not properly placed inside the carriage. There is no buffer in the tank.**
   - **The buffer is too diluted.**
   - **The photo hood is not on the carriage. There is too little running buffer.**
   - **The power supply is not plugged in. Check by turning on the blue LEDs.**
   - **If the green power LED is blinking, please refer to the troubleshooting steps in the MiniOne Electrophoresis Instruction Manual**



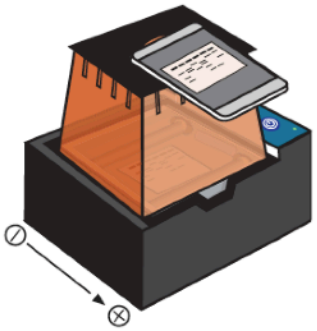
2. Have students periodically check the migration of the bands (~every five minutes).



3. Allow the gel to run **25 minutes** or until DNA separation is sufficient. Keep in mind small DNA samples run faster so it's important to periodically check where your bands are. After your run is complete, turn off the power by pressing the button. Use the low intensity for viewing during the run. Light will weaken the fluorescent DNA signal.

4. Document your results.

   Wipe off the condensation from the inside of the hood with a soft cloth if necessary, then place the hood back on the carriage. **Turn on** the high intensity light. Place your cell phone or camera directly on the photo hood to take a picture of the DNA. **DO NOT** zoom in as this will result in blurry pictures. (The photo hood is already at the optimal focal length for a smart device.



5. Clean up. Follow teacher's instructions on disposal and clean up.

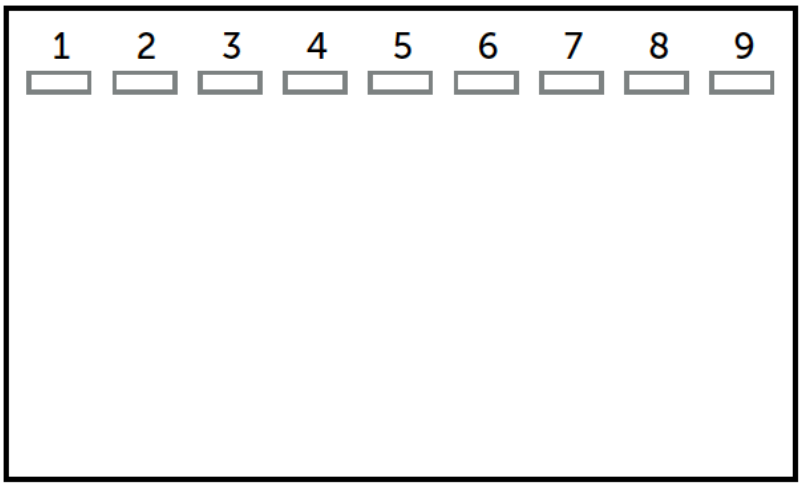
Clean Up

Note: All reagents in this lab can be disposed of as non-hazardous waste.

1. After collecting data and documenting results, remove the photo hood and unplug the power supply from the wall and from the back of the MiniOne® Carriage Remove the clear running tank from the carriage and remove the gel and tray from the running tank.
2. Pour the used running buffer down the drain or into a waste beaker. Throw the gel away AND SAVE THE GEL TRAYS. Rinse the clear plastic running tank, gel tray, comb, and casting system with DI or distilled water. Allow the tanks to fully air dry before storing.
3. Use a paper towel or Kimwipe™ to gently wipe the gold rivets in the carriage (where the electrodes connect) to ensure all moisture is removed. Wipe up any buffer that may have spilled into the black carriage. Follow any additional directions the instructor gives for clean up and storage.

Part II: Results

What does your gel look like? Record images of the gel in the gel below



Lane 1: _____
Lane 2: _____
Lane 3: _____
Lane 4: _____
Lane 5: _____
Lane 6: _____
Lane 7: _____
Lane 8: _____
Lane 9: _____

Post Lab Analysis

1. The DNA for genetic tests is usually obtained from a blood sample or from a cheek swab or saliva sample. **Explain** why these would be used as the source of DNA for a genetic test?

2. Red blood cells usually lose their nuclei as the cells mature, so what type of cell would be used to provide DNA from the blood sample?

3. The DNA sample obtained from the blood sample, cheek, or saliva will only contain a small amount of DNA and we only need copies of a specific gene. What process could we use to make many copies of a desired gene?

4. How does gel electrophoresis separate DNA fragments?
    a. What **causes** the DNA to be cut into fragments?

    b. What charge is the DNA and why does that charge and the gel chamber/power source cause the DNA to move?

    c. How do the small fragments move compared to the larger fragments?

    d. What is the purpose of the buffer solution in the chamber?

Meeting with the Genetic Counselor

"Mr. and Mrs. Thomas, I am Dr. Hughes. I am here to help you understand the results of the genetic screening we performed. Let's first discuss the inheritance and probability of FH.

Since both of you have been confirmed to have HeFH, let's complete a Punnett Square to show you the probability of you passing the condition on to your children."

Develop a mathematical model (i.e., Punnett Square) for Jada and Jared's possible offspring. Show the probability of the couple having a child without FH. What is the probability that they would have a child with HeFH? What is the probability that they would have a child with HoFH?

"There are three genes that are usually used for genetic screening for FH. Those genes are *LDLR*, *APOB*, and *PCSK9.* One of the DNA variants or mutations does appear to be present in your family, specifically for the low-density lipoprotein receptor protein coded for by the LDLR gene. The variant of the gene you possess either results in too few of these protein receptors or proteins that do not function properly. These receptors are supposed to help take up the lipoprotein for LDL in the liver so it can be removed from the bloodstream. As we mentioned in the consultation prior to the genetic screening, a negative test does not completely rule out a possible FH diagnosis. We are still learning about alternative genetic causes and there can be technical limitations of the test. For these reasons, it is important to monitor LDL cholesterol levels in all the individuals, even those who did not possess the mutated/variants of the gene."

"I cannot begin to describe how overwhelming this experience has been, but we appreciate all the information and guidance all of you are providing. Now that we have the results, what options do we have? Should others in our family be tested as well?" asked Jada.

"I am going to provide you with some statistics from the National Organization for Rare Disorders. I don't want to alarm you but I want you to understand the seriousness of the condition. The good news is that there are preventive measures and treatments. Are you ready to discuss that information?" Dr. Hughes asked.

Jada nodded and Jared agreed, "Yes."

"According to the National Organization for Rare Disorders, '[having] HeFH greatly increases the risk of hardening of the arteries (atherosclerosis), which can lead to heart attacks, strokes and other vascular conditions. Untreated individuals with FH have a 20-fold increased risk for coronary artery disease (CAD). Untreated men have a 50% risk of a nonfatal or fatal heart attack (coronary artery blockage) by age 50 years; untreated women have a 30% risk by age 60 years. If one or more other risk factors for CAD are present, especially cigarette smoking or diabetes mellitus, the risk of developing symptomatic CAD is even higher.'

While those statistics seem very scary, there are preventative steps that can be taken. The good news is that you detected the condition. For many, this condition goes unnoticed until coronary artery disease has already progressed. With medication known as statins, other potential

medications, and lifestyle changes in diet and exercise, you can treat your condition and reduce the risk of cardiovascular disease. It will be important for you to discuss a plan with a specialist in cardiovascular disease. Lifestyle changes alone are not usually enough to treat this genetic condition," Dr. Hughes explained.

"That addresses us, but what about Amber?" asked Jared.

"Since Amber has HoFH, her treatment will need to be much more aggressive. Without treatment, she can develop coronary artery disease and aortic stenosis which is a narrowing of the aortic valve opening that allows oxygenated blood to be pumped from the left ventricle to the aorta and the rest of the body. Without aggressive treatment, the likelihood that someone will die with HoFH before the age of 30 is high. There are medications specific to HoFH and even a process called LDL apheresis that helps to remove the LDL from the blood plasma using a machine. Amber also has a 100% chance of passing the mutated version of the gene to her children even if her husband is unaffected. They would at least have HeFH," explained Dr. Hughes.

"And what about the rest of our family, like Jada asked before?" said Jared.

"Now that we have these results, it is important for you to inform other family members. There is an option known as cascade screening or family screening. This can be DNA testing or testing for LDL cholesterol levels. This is basically where we test close relatives in a stepwise fashion. We could then do a pedigree analysis of your family. These tests could uncover other family members who are at risk so they can seek treatment as well.

Jada and Jared looked at each other. They had experienced a roller coaster of emotions over the last few weeks as they learned more and more about FH, but they were prepared to face it and to meet with the cardiovascular specialist to develop a treatment plan for themselves and for Amber.

"We are ready for the next step," said Jada.

Analysis Questions:

1. If you were Jada or Jared, what specific **questions** (at least 2) would you **ask** the genetic counselor?

2. Why does the DNA variant of the LDLR gene **cause** higher levels of LDL cholesterol in the bloodstream (**effect**)? Why are the levels of LDL cholesterol even higher (**effect**) for someone with two copies of the defective gene (**cause)**?

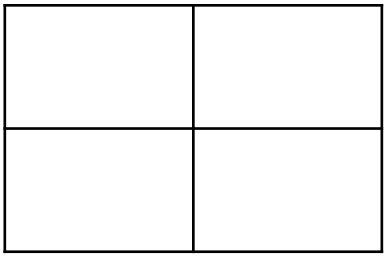
3. Why does increased LDL cholesterol in the bloodstream (**cause**) increase a person's risk of coronary artery disease and myocardial infarction (heart attack) (**effect**)?

4. What other effects could FH have on the human body that could put the affected person's life at risk?

5. Jared is curious about why medication is needed. **Construct an explanation** of how you would explain statins to Jared and Jada and how they work to lower LDL cholesterol levels in the bloodstream.

6. Pretend you are a cardiovascular specialist who will help Jada and Jared come up with a treatment plan for them and for Amber. Create a table listing the treatments you would advise and why that treatment is needed. Your plan should include medications, lifestyle changes (i.e., diet, exercise) and other possible treatments. Keep in mind that the treatment for HeFH and HoFH will not be exactly the same.

7. The genetic counselor said that Amber has a 100% chance of passing on the defective allele/variant for the LDLR gene. Complete a **mathematical model** (i.e., Punnett Square) that provides **evidence** for that **claim**.



8. What might **cause** a person with HoFH to need a liver transplant? Do the risks associated with a liver transplant outweigh the risks associated with HoFH?

Appendix A - Research Extensions

AP Biology

1. Unit 1 Connection: Research the mechanisms behind cholesterol metabolism, synthesis and homeostasis.
2. Units 2 and 4 Connection: Research a signal transduction pathway related to cholesterol use and uptake within the cell for normal levels and functioning. Find a pathway that shows how those levels can be impacted by FH.
3. Unit 5 Connection: Research the genetics of FH in more detail.

Biology/AP Biology (Unit 7 Connection)

1. Research why there are higher incidence levels of FH in certain subpopulations throughout the world like Lebanese Christians, Afrikaners in South Africa, French Canadians, and Ashkenazi Jews originating from Lithuania.
2. There is evidence that the incidence of FH in Ashkenazi Jews originating from Lithuania is 1 in 67 due to a founder effect. Bottleneck events may have also been responsible. Research these forms of genetic drift and try to determine why these mechanisms of evolution may have resulted in the higher incidence. What might have caused these events from a historical perspective and how did it impact the subsequent generations?

Appendix B - References

Information from the following sources was used to write this case study.

NORD - Rare Disease Database: Familial Hypercholesterolemia

Healthline: What is Xanthoma?

Science Direct: Xanthoma

National Library of Medicine: Corneal arcus and xanthomas in homozygous familial hypercholesterolemia: First report from China

National Library of Medicine: A Population-Genetic Test of Founder Effects and Implications for Ashkenazi Jewish Diseases

National Library of Medicine: Tendinous xanthoma with familial hypercholesterolemia

Case Reports in Orthopedics: A Rare Case of Bilateral Achilles Tendon Xanthomas in a Teenager, Successfully Treated with Tendon Sparing Technique

Monitoring Blood Glucose

University of Rochester Medical Center: Cholesterol, LDL, HDL, and Triglycerides in Children and Teens

Journal of Cutaneous and Aesthetic Surgery: Giant tuberous xanthomas in a case of type IIA hypercholesterolemia

UC San Diego Health: LDL Apheresis

American Heart Association: Familial Hypercholesterolemia (FH)

CDC: Genetic Testing for Familial Hypercholesterolemia

miniOne®
SYSTEMS

theminione.com (858) 684-3190 info@theminione.com